IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	)	
MESSADEK, Jallal	)	
Application Ser. No. 09/945,391	)	Group Art Unit: 1614
Filed: August 31, 2001	)	
Glycine betaine and its use	)	Examiner: Clinton T. Ostrup

DECLARATION OF JALLAL MESSADEK PURSUANT TO ART 37 C.F.R. 1.132

1. I, Jallal Messadek, a citizen of Belgium and residing in Liege, Belgium, make the following declaration in support of the above-referenced patent application filed and now pending in the United States Patent and Trademark Office. I am over the age of eighteen, under no disability, and fluent in English and understand the following statements made in support of this application.
2. I carefully read the Office action and have the following comments to make.
3. The claims have been amended so as to take into account examiner's remarks.
4. For example, claim 1 has been amended so as to specify:

- that the method is directed for treating human at risk to suffer from thrombosis of arterial or venous origin ;
- that the patient is a human ;
- that the effective amount of glycine betaine is administered daily ; and
- that the administration is carried out by oral dosage form, subcutaneous injectable dosage form and patches.

5. Claim 4 has been amended so as to specify that the method is directed for treating human at risk to suffer from thrombo-embolic diseases of arterial or venous origin not induced by hyperhomocysteinuria or homocystinuria, that the effective amount of glycine betaine is administered daily, that glycine betaine is used as antithrombotic agent, and that the administration is carried out by oral dosage form, subcutaneous injectable dosage form and patches. Paragraphs 0002, 0016, 0190 and 0137 of the description give the support for this amended claim. Thrombo-embolic disease is a technical term obvious for the skilled man. For this, please compare passage from my description, paragraph 0016:" *Thrombo-*

embolic disease of arterial or venous origin remains one of the main reasons of death in developed countries" with Da Silva MS & Sobel M. document [Anticoagulants: to bleed or not to bleed, that is the question. Semin Vasc Surg. 2002 Dec; 15(4):256-67 SUNY Upstate Medical University, Syracuse, NY, USA] first phrase: "*Thromboembolic vascular diseases remain the main cause of death in Western industrialized societies*". (Exhibit 1)

6. Claim 7 has been amended so as to specify that the method is directed for treating human at risk to suffer from blood coagulation disorders, that glycine betaine is administered daily, that glycine betaine is used as antithrombotic agent, and that the administration is carried out by oral dosage form, subcutaneous injectable dosage form and patches.

7. Claim 12 has been amended so as to specify that the method is directed for treating human at risk to suffer from thromboses and with hemorrhage risk, that glycine betaine is administered daily, that glycine betaine is used as antithrombotic agent, and that the administration is carried out by oral dosage form, subcutaneous injectable dosage form and patches.

8. Claim 15 has been amended so as to specify that glycine betaine is used as lysing agent and that that the administration is carried out by oral dosage form, subcutaneous injectable dosage form and patches.

9. Claim 22 has been amended so as to specify that glycine betaine is administered as counteracting agent against thromboembolic effects induced by the administrated contrasting agent, and that the administration is carried out by oral dosage form, subcutaneous injectable dosage form and patches

10. Claim 28 has been amended so as to specify that glycine betaine is used as lessening agent of the incidence of hemorrhagic side effects due to the administration of the antithrombotic active agent other than glycine betaine, and that the administration is carried out by oral dosage form, subcutaneous injectable dosage form and patches.

11. New claims have been added for method of treatment of human suffering or at risk to suffering from specific troubles.

12. With respect to the remarks of the examiner about the broad claims of my application, I want to draw the attention on the fact that the claims have been amended, but moreover that the pending claims are well supported by various well admitted tests carried out by eminent Professors who have confirmed the anti thrombotic activity of glycine betaine. The digest of these tests is disclosed in the brochure attached to my first affidavit.

As it appears, glycine betaine is effective against different experimental thrombosis in different species, namely: the laser induced thrombosis, the venous thrombosis induced by stasis and Ferric Chloride topical application induced thrombosis performed under my supervision. Betaine antithrombotic effect was also evaluated by tests performed externally, namely: Induced focal cerebral ischemia by Professor Christoph de HAEN (63 International publications), and free radical induced femoral artery thrombosis by Professor Marc HOYLAERTS (90 International publications). For the skilled expert, these different animal approaches carried out with betaine are well-known and recognized to be predictive of therapeutic human efficiency of compounds candidate to be future anti thrombotic drugs.

The skilled man considers that only one of these tests is already well predictive of a molecule antithrombotic efficacy in human. Many established antithrombotic drugs and some approved medicines did not show, nor claim as many of these experimental different settings. Glycine Betaine showed a sound antithrombotic effect in these different various models witch was externally confirmed and further validated on in vitro tests on human blood:

- In a flow perfusion device system, witch dynamically reconstitutes blood flow conditions inside vasculature, Professor Giuseppe REMUZZI (567 international publications) demonstrated on human blood that betaine was active either on collagen and endothelial cells i.e. that betaine possesses both anticoagulant and anti-aggregant properties, said properties allowing it to be effective in different pathological conditions.

I have shown in my first affidavit that glycine betaine has a very low toxicity, namely:

10800 mg/kg in case of oral administration, and

830 mg/kg in case of parenteral administration.

The treatment according to my invention is thus safe and efficient as shown by tests made by eminent professors.

As a same standard has to be used for all granted US patents, my pending claims are well supported.

I made a quick search among recent granted US patents and I refer as example only to the following specific patents: US 6,613,328; US 6,617,359; US 6,624,180, said patents being granted with very broad claims.

Indeed, claim 1 of US 6,613,328 relates to:

"A method of treating a patient having or at risk of a thrombotic disease or atherosclerosis, comprising: administering to said patient an effective dose of a humanized immunoglobulin, which comprises the heavy chain variable region SEQ ID NO:5 and the light chain variable region SEQ ID NO:7".

Said claim does not refer to the form of administration, while being also directed towards the treatment and the prevention of thrombosis. In the specification of this granted patent, reference is made to a unique in vitro binding test (example 4) used for determining biological activity of the claimed compounds.

Claim 13 of US 6,617,359 relates to:

"A method of treating a mammal suffering from a disease chosen from stable and unstable angina pectoris, coronary heart disease, Prinzmetal angina, acute coronary syndrome, heart failure, myocardial infarction, stroke, thrombosis, peripheral artery occlusive disease, endothelial dysfunction, atherosclerosis, restenosis, endothelial damage after PTCA, hypertension, chronic glomerulonephritis, erectile dysfunction, ventricular arrhythmia, diabetes and diabetes complications, nephropathy and

retinopathy, angiogenesis, asthma bronchiale, chronic renal failure, cirrhosis of the liver, osteoporosis, restricted memory performance, and a restricted ability to learn, which method comprises administering a physiologically active amount of 4-fluoro-N-indan-2-yl benzamide according to the formula (I) ##STR5## to the mammal, wherein the physiologically active amount of 4-fluoro-N-indan-2-yl benzamide stimulates the expression of endothelial NO-synthase in the mammal."

Such a claim covers various diseases, among which thromboses, with no indication of the administration path. In the specification of this patent reference is made to a single in vitro test and four animal in vivo tests for claiming a method of treatment for practically the half of the known human diseases.

Claim 23 of US 6,624,180 relates to:

"A composition for inhibiting thrombotic conditions in blood comprising a compound of claim 1 and a pharmaceutically acceptable carrier."

Claim 1 of US 6,624,180 relates to a family of compounds, i.e. more than a few hundred different compounds. In this specification only 4 in vitro tests are made for determining biological activity of 14 compounds out of hundreds (if not thousands) claimed. These in vitro tests were used only to determine background absorbance of some compounds, and were not using biological material such as blood, blood constituents or endothelial cells which are the main actors of the claimed pathologies.

The predictability for these hundred compounds was thus admitted by the USPTO for inhibiting thrombotic conditions, i.e. treating and/or preventing thromboses. (See "***FIELD OF THE INVENTION***

This invention is in the field of anticoagulant therapy, and specifically relates to compounds, compositions and methods for <u>preventing</u> and treating thrombotic conditions such as coronary artery and cerebrovascular disease."

In view of the numerous tests made for my invention, and in view of the standards accepted for granting US patent, my application completely fulfils USPTO's predictability standards.

13. I have added a new claim 31 for a method of treating thromboses not induced by hyperhomocysteinuria or homocystinuria in a human, this claim being a splitting of claim 1.

14. With respect to new claim 32 relating to a method of treating a human suffering from blood coagulation disorders, said claim has been added as a splitting of previous claim 7 which relates to a method for treating patient at increased risk of suffering blood coagulation disorders, as well as for treating patient suffering blood coagulation disorder.

15. I added a new claim 42 for a method of treating human at increased risk to suffer from blood coagulation disorders as defined by Virshow. Reference to Virshow is specifically made in page 4 of the present application.

16. I added also several new claims for specific methods, namely

- claim 47 for a method of treating a human suffering from thrombin formation
- claim 48 for a method of treating a human suffering of a blood hypercoagulable state as defined by Virchow
- claim 49 for a method of treating a human at risk to suffer of a blood hypercoagulable state as defined by Virchow
- claim 50 for a method of treating patient at risk to suffer from blood coagulation activation
- claim 52 for a method of treating humans suffering from a disease selected from the group consisting of thrombosis, thrombo-embolic diseases, blood coagulation activation disorders
- claim 53 for a method of treating humans suffering from thrombin formation
- claim 54 for a method of treating humans at risk to suffer from a disease selected from the group consisting of thrombosis, thrombo-embolic diseases, blood coagulation activation disorders
- Claim 55 for a method of lysing thrombus in a human for a method of treating humans suffering from a disease selected from the group consisting of thrombosis, thrombo-embolic diseases, blood coagulation activation disorders.

17. With respect to the Mar et al reference cited in the Office Action, I want to make the following remarks:

Mar et al discloses that Betaine is present in wine at a rate of 3 mg per glass.

In my experiments as in the experiments conducted on animals by Professor Marc Hoylaerts, as in those in vitro on human blood conducted by Professors HC. Hemker & G. Remuzzi, betaine antithrombotic effect appears since a concentration of 20 μg/ml in blood, which represents a threshold for its pharmacological activity. These studies point a concentration around 30 μg/ml to be the IC_{50} of betaine antithrombotic effect. A therapeutically effective amount (IC_{50}) of betaine in a normal person must be at least 150 mg in blood per body (30 μg x 5000 ml blood) if the bioavailability of betaine is 100%. Wine is ingested orally and attached exhibit 2 teaches that betaine absolute oral bioavaibility is around 11% i.e. when one drinks one glass of wine containing 3 mg of betaine only 330 μg reaches his blood. It means, according to Mar's document, that one have to drink more than 450 glasses of wine to achieve an antithrombotic blood concentration of 30μg/ml.

It means also that for administering a daily dose of 5 g (i.e. 550 mg reaching the bloodstream) as in example 21 of the present patent application, it would be necessary that the human drinks more than 1,650 glasses of wine. Even if the daily effective antithrombotic dose could be reduced by 100 times, it still represents 3 bottles of wine daily.

Mar et al does therefore not teach, nor suggest the method of my invention. By applying the Mar et al wine drinking method, it will not be able to achieve an effective therapeutic amount of glycine betaine as anti thrombotic agent, as anticoagulant agent, etc. without killing the patient due to alcohol overdose.

18. With respect to the Folia Medica reference cited in the Office Action, I want to make the following remarks:

Folia medica teaches that the simultaneous administration of betaine, choline, and vitamin B12 has a protective effect on the prolongation of blood coagulation due to an administration of p-dichlorobenzene and olive oil in guinea pigs.

This document does not teach a method with a daily administration of an effective therapeutic amount of glycine betaine. Folica medica determines the appearance of the disturbance of blood coagulation in the diagnosis of p-dichlorobenzene intoxication. If the purpose is to determine blood coagulation disturbance due to p-dichlorobenzene injection, the simultaneous administration of betaine, choline and vitamin B12 would have a protective effect against p-dichlorobenzene.

Folia Medica does not teach that betaine per se has an antithrombotic efficiency, nor that a daily administration is required. Furthermore, my invention relates to method for treating human for example being at risk of thrombosis, not to a method in which a poison (p-dichlorobenzene) is subacutely administered to animals.

Folia medica does not teach, nor suggest that glycine betaine is an agent for reducing the activation of constituents of blood and for reducing the activation of coagulation resulting in the formation of thrombin.

19. With respect to new claims 42, 48 and 49, referring to Virchow, I want to make the following remarks:

The aim of any antithrombotic drug is to reduce the manifestation of at least one of the Virchow's Triad parameters namely: haemodynamic and rheological factors, endothelial lesion and activation of the constituents of blood, particularly of platelets, and of coagulation which results in thrombin formation and hypercoagulable state. (see Pubmed, J Vasc Surg. 2000 Feb; 31(2): 396-405, "the caput medusae of hypercoagulabity" by Silver D et al, see also W V Med J. 1993 Feb; 89(2):61-3 "Hypercoagulable states" by Rogers JS; Langenbecks Arch Chir 1986;369:451-7 "Principles of drug prevention of thrombosis" by Lasch HG; Ric Clin Lab 1983; 13 Suppl 3:71-81 "from Virchow to Red cells (the unended quest)" by Marcel GA et al, and Semin Thromb Hemost. 1991;17 Suppl 3:250-3 "Pathophysiology of venous tromboembolism" by Nielsen HK). In the specification thrombosis was defined according to Virchow's Triad and betaine antithrombotic activity as reducing blood coagulation activation resulting in thrombin formation. Thrombosis manifestation encompasses hypercoagulable state and in any case Folia Medica does not teach that betaine, choline and vitamin B12 reduce one of the parameters of Virchow's Triad, but on the contrary Folia Medica teaches that betaine, choline and vitamin B12

provide a protective effect against prolongation of blood coagulation i.e. hypocoagulation induced by p-dichlorobenzene and olive oil subacute poisoning.

20. With respect to Haussinger and to Kalvinish references cited in the Office action, I want to address the following remarks:

Ischemia is a consequence of thrombosis, whereby when treating a consequence there is no teaching of a treatment of the causes of said consequence. For any expert in the field, no direct and obvious link can be made between a method for treating a consequence and a method for treating the causes. For example, when a person suffers from a cough or influenza, fever is treated with an active agent for relieving their consequences (pain, fever, etc.), said treatment is not effective against the viral infection causing said troubles or consequences, i.e. pain, fever, etc.

Haussinger teaches only (see column 3, lines 40 to 43 and claim 1) the use of an effective amount of an osmolyte in the preparation of a therapeutic agent capable of treating or preventing complications resulting from ischemia, hypoxia or oxidative stress.

In column 4, lines 58 to 62, Haussinger teaches a method of treating complications resulting from ischemia, hypoxia or oxidative stress with a composition comprising therapeutically effective amounts of an osmolyte **and** a thrombolytic agent. It is this mixture that will be useful for treating complications in relations to myocardial infection wherein the thrombolytic agent with a capacity to induce lysis of blood clots. The osmolytes, such as glycine betaine, are used as agents in the regulation of the cellular hydration with the additional capacity to protect organs against injuries resulting from ischemia, hypoxia and oxidative stress, i.e. for treating or preventing complications resulting from ischemia, hypoxia and oxidative stress.

Haussinger does not teach, nor suggest that glycine betaine per se (Haussinger proposes to use a mixture of an osmolyte with a thrombolytic agent, meaning thus that the osmolyte is in no way a thrombolytic agent, nor possesses per se any property to induce lysis of blood clots) can be used as an anti thrombotic agent, anti coagulant agent, clot lysing agent, etc. Haussinger teaches away from my invention, as according to Haussinger the osmolyte can be used for treating or preventing complications resulting from ischemia, while my invention teaches that glycine betaine can be used,as such, as antithrombotic agent, for

example as agent suitable for preventing the occurrence of pathological events i.e. thrombosis leading to ischemia as such. Haussinger teaches how to treat or to prevent the consequence, while my invention teaches how to treat or to prevent the cause.

Kalvinh discloses a pharmaceutical composition containing Gamma-Butyrobetaine. Gamma butyro betaine is a compound which can not be considered as equivalent to glycine betaine. The difference existing between glycine betaine and gamma butyro betaine has already been questioned in the preliminary examination of PCT/BE 00/00021. Said tests are disclosed in my first affidavit.

As gamma butyro betaine is in no way equivalent to glycine betaine, the combination of Haussinger with Kalvinh will in no way disclose my method of treatment.

Furthermore, it has to be mentioned that betaine antithrombotic activity was not obvious for the skilled experts. Upon my request, its tests had been screened and validated, using different experimental models, by eminent independent experts who have a sharp knowledge of what is the state of the art in their scientific field and often contributing by their works to establish and to perfect said state of the art (1094 International publications available on PubMed). If betaine antithrombotic effect was so obvious, the work done by these 5 eminent Professors could have been a loss of time for them with no particular scientific interest, and they would advise me to abstain to follow an evident & intellectually unprofitable way. It was not the case.

21. As I receive from the Chinese patent Office a document in Chinese, I ordered an English translation thereof, in order to better know the content thereof.

A copy of said Chinese document, as well as an English translation thereof is attached hereto. As it appears from the English translation of said Chinese document, said document is silent to glycine betaine anti thrombotic properties and is therefor not pertinent for my invention.

22. I hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that

these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Done at Liege, November 6th, 2003

Jallal Messadek